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Mae B. Hultin, M.D.

Fibrinogen and Factor VII as Risk Factors in Vascular Disease

INTRODUCTION

Several prospective epidemiologic studies have demonstrated a positive statistical correlation of the plasma fibrinogen concentration and the plasma factor VII level with subsequent ischemic cardiovascular events, including acute myocardial infarction, sudden death and ischemic cerebrovascular accident. For fibrinogen, this correlation was statistically significant for ischemic cardiac events in both men and women (54,66,94,101) and for cerebrovascular accidents in men (54,101). For factor VII, a significant association with subsequent ischemic cardiac events was found in men (66). In most cases, multivariate analysis showed the fibrinogen or factor VII level to be a significant risk factor after adjusting for other known risk factors, such as blood pressure, smoking, blood lipid levels and diabetes mellitus.

Since these studies in the United States and Europe involved observation of large numbers of individuals over many years, it is likely that their findings can be extrapolated to the general population living in similar societies. However, certain limitations should be noted. Only two studies included female subjects (54,66), with only the latter reporting significant findings in women, suggesting the need for further studies of these possible risk factors in women. Furthermore, the sole study that included measurement of factor VII as a possible risk factor (66) has yet to be confirmed, although preliminary findings from another ongoing study also support an association of factor VII levels with ischemic cardiac events (5). It

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is important, therefore, that the specific design and principal findings of these studies be analyzed in detail. These intriguing associations raise important questions concerning the potential mechanisms by which the elevated protein levels influence these pathologic conditions. It is therefore appropriate to analyze some of the other studies, including cross-sectional clinical and biochemical studies, aimed at clarifying the role of these proteins in ischemic vascular disease. As background for understanding the epidemiologic studies, it is appropriate to review briefly the biochemistry of these proteins. For a detailed discussion of the structure and function of fibrinogen, an extensive review is available (43); another recent review discussed at length the possible mechanisms by which fibrinogen may be involved in atherogenesis (93).

BIOCHEMISTRY OF FIBRINOGEN

Fibrinogen is a large plasma glycoprotein with a molecular weight of 340,000, which is formed from three pairs of polypeptide chains known as the A-alpha, B-beta, and gamma chains (Table 7–1). Thrombin cleaves the small amino-terminal fibrinopeptides A and B from the parent polypeptide chains, producing a soluble fibrin monomer. These monomers then associate non-covalently into fibrin polymers, which are subsequently cross-linked covalently by activated factor XIII to form insoluble fibrin (78). Fibrinogen itself can also be cross-linked directly by activated factor XIII (51). The genes for all three chains of fibrinogen are located on the long arm of chromosome 4 (55). Fibrinogen is produced by the liver and is relatively abundant in plasma, with a normal level of 2–4 grams per liter, and a half-life of 3–4.5 days (19). Fibrinogen is also synthesized by megakaryocytes and is present in the alpha granules of platelets. Fibrinogen binds specifically to activated platelets via the membrane glycoprotein IIb/IIIa, resulting in platelet aggregation. Platelets are also incorporated into and influence the characteristics of the fibrin clot. Some fibrinogen (10 to 25 percent of the total pool) is distributed extravascularly in lymphatic and interstitial fluid and may have important functions in extravascular processes such as inflammation. Since fibrinogen is an acute-phase reactant, its plasma level can increase substantially in a number of settings, both physiologic and pathologic (103). Fibrinogen degradation products produced by the action of plasmin exert a feedback control on fibrinogen synthesis via the action of hepatocyte stimulating factor, a monocyte-derived polypeptide (7,85).

Table 7–1. Characteristics of Fibrinogen and Factor VII

	Fibrinogen	*Factor VII*
Molecular weight	340,000	56,000
Chain composition	3 pairs of Aα Bβ, and γ	Single-chain
Plasma concentration	2–4 g/L	300–600 μg/L
Plasma half-life	3–4.5 d	5–6 hr
Site of synthesis	liver, megakaryocyte	liver
Gene location	chromosome 4	chromosome 13

Fibrinogen also binds to other plasma proteins, including thrombospondin and fibronectin, both of which are incorporated into plasma fibrin clots. Thrombin binds to fibrin clots and retains its activity when bound, providing a means for the localization of thrombin at the site of clot formation. Since fibrinogen is an abundant and asymmetric plasma protein, increases in its concentration increase the blood viscosity and have rheologic implications (58), apart from the specific interactions of the protein in fibrin clot formation. Thus there are many potential mechanisms by which fibrinogen might function as a risk factor for ischemic vascular events, including fibrin formation, platelet aggregation, blood rheology, and inflammation.

BIOCHEMISTRY OF FACTOR VII

Factor VII is a trace plasma glycoprotein that is a member of a group of homologous proteins synthesized by the liver (Table 7–1). All members of the group undergo a post-synthetic modification (gamma-carboxylation of glutamic acids) requiring vitamin K as a cofactor. Other proteins of this group—factors IX, X, and prothrombin—are also procoagulants (78) and share a number of structural homologies. Other members of the group (proteins C and S) have anticoagulant properties. The complete sequence of human factor VII has been determined from the cDNA sequence (40). The genes for factors VII and X are located near the end of the long arm of chromosome 13 (81), and evidence from a patient with a deletion in the short arm of chromosome 8 suggests there may be a regulator gene in that region (27). Factor VII is present in plasma in very small amounts, with a normal level by radioimmunoassay of approximately 470 micrograms per liter (28). It is a single-chain polypeptide of 406 amino acids (56,000 molecular weight) which when activated by factor Xa is converted to a two-chain enzyme form (83). Factor XIIa and IXa can also activate FVII in vitro. The two-chain form of factor VII activates factor X slowly in the absence of tissue factor but is markedly potentiated by its presence.

A diagrammatic scheme of the generation of factor Xa is shown in Figure 7–1. For the sake of simplicity, some of the complex interactions between the extrinsic and intrinsic pathways have been omitted, as well as several feedback reactions, such as activation of factors VIII and V by thrombin. Some evidence supports the ability of the single-chain form of factor VII to catalyze slowly the cleavage of factor X in the presence of tissue factor (104), but other studies disagree with this conclusion (102). If this is operationally true *in vivo* (i.e. that the circulating single-chain form is not a completely inactive zymogen but has some weak enzymatic activity), it could provide a theoretical construct for the particular importance of factor VII in initiating clot formation at a site of exposed tissue factor. In general, stimuli such as pregnancy or estrogen administration that increase factor VII also increase IX, X, and prothrombin (103), and thus the latter factors may be involved in the overall effect associated with the elevation of factor VII. Moreover, there may be additive or synergistic effects of elevations of these factors, leading to increased generation of factor Xa by both the extrinsic and intrinsic pathways and ultimately increased generation of thrombin (Figure 7–1).

The half-life of factor VII *in vivo* is very short at approximately 5-6 hours

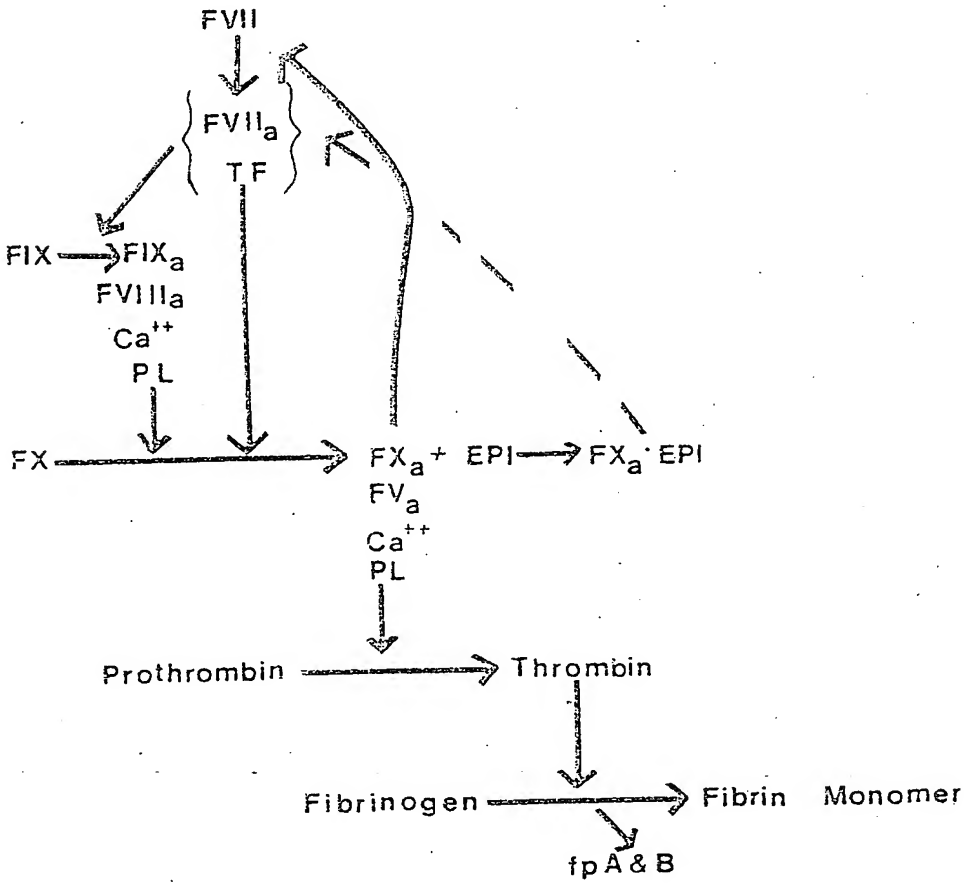

Figure 7-1. Coagulation pathways leading to fibrin formation. PL = phospholipids or platelets, EPI = extrinsic pathway inhibitor, fp = fibrinopeptide.

(45), and that of factor VIIa is even shorter, about 2 hr (89). A small amount of factor Xa, generated by any route, can produce the subsequent amplification of the extrinsic pathway of coagulation by activating factor VII (Figure 7–1). Antithrombin III has little or no inhibitory activity against factor VIIa, and until recently it was widely believed that there was no physiologic plasma inhibitor of factor VIIa. Recent studies have found a lipoprotein-associated coagulation inhibitor, or extrinsic pathway inhibitor (see Chapter 8, in this volume), which in the presence of factor Xa inhibits the activity of factor VIIa/ tissue factor (Figure 7–1) (8). Unlike the diverse functions ascribed to fibrinogen, no functional role for factor VII other than its involvement in factor X activation has been reported. The assay methods used for factor VII, as well as for fibrinogen, will be discussed in the analysis of each study.

TERMINOLOGY

In citations in this review, the terminology used by the investigators cited will be used, since their choice generally was tailored to the specific characteristics of their subjects or to the endpoints under study. For instance, the Framingham study used the term cardiovascular disease to encompass 4 types of end-points or events, including stroke and myocardial infarction. The term coronary artery disease has been used to describe patients who had proof of infarction while in other cases it may be applied to patients who only had angiographically proved disease. Some investigators use the term ischemic heart disease to indicate subjects who have had ischemic symptoms or events due to coronary artery disease. Thus it would be difficult and potentially inaccurate to superimpose a uniform terminology on all the studies cited.

EPIDEMIOLOGIC STUDIES OF HEMOSTATIC VARIABLES AS RISK FACTORS FOR MYOCARDIAL INFARCTION AND STROKE

The Northwick Park Heart Study was the first prospective epidemiologic study to report significant associations between the subjects' plasma factor VII and fibrinogen levels at the time of entry into the study and the subsequent occurrence of ischemic cardiac events (64) (Table 7–2). Two smaller studies, from Gothenberg, Sweden, and Leigh, England, only concerned fibrinogen, and these will be discussed first.

The Gothenberg Study

This prospective epidemiologic study found a significant association between fibrinogen level at entry and subsequent ischemic vascular events (101 and Table 7–2). In this study, all men born in 1913 in Sweden and living in Gothenberg in 1963 were identified from the Swedish national registry system. Birth dates defined the eligible pool; of these, 792 men (81 percent) agreed to participate and were examined in 1967. The parameters studied at entry included total serum cholesterol, systolic blood pressure, smoking status, plasma fibrinogen (by the clotting method of Blombäck), clotting factors II-VII-X (the P and P test), factor VIII, fibrinolytic activity, and plasminogen, with blood drawn in the afternoon after a 6–8 hour fast. The reported analysis of outcomes (myocardial infarction, stroke and death from all causes) was based on 13.5 years of observation of all 792 participants by a combination of interviews, reviews of hospital records, citywide registers for myocardial infarction and stroke using standardized criteria, review of all death certificates, and autopsies. The autopsy rate was 100 percent in men dying out of hospital and greater than 80 percent overall. These are impressive achievements in data collection. Although the sample size is somewhat smaller than optimal for prospective studies (see below), the impressively complete data collection, including pathologic verification, should maximize the accuracy of endpoint determination and compensate for the relatively small sample size. The sampling method for

Table 7–2. Characteristics of Prospective Studies of Coagulation Factors and Risk of Ischemic Cardiovascular Disease

Study (Ref.)	*No. and Sex of Subjects**	*Age (yr) At Entry*		*No. Subjects Evaluated With Endpoint/ Without Endpoint*	*End-Points#*	*Follow-Up Interval (yr)*		*Significant Association With Endpoint*
		Mean	*Range*			*Mean*	*Range*	
Northwick Park: (64,66)	1,511	52	40–64	1) 68/1443	1) Death From Ischemic Heart Disease	1) 10.0	7.3–13.5	1) Fibrinogen Factor VII
				2) 60/1399	2) Non-Fatal Myocardial Infarction	2) 6.7	2.1–12.6	2) Fibrinogen Factor VII^Δ
Gothenberg: (101)	792 Males	54†	–	1) 37/755 2) 92/700	1) Stroke 2) Myocardial Infarction	13.5†	–	1) Fibrinogen 2) Fibrinogen
Leigh: (94)	297 Males	52.4	40–69	1) 40/257	1) Myocardial Infarction	7.3	0.1–16.1	1) Fibrinogen
Framingham: (53,54)	554 males 761 females	>55	47–79	1) 117/437 in males 97/664 in females 2) 92/1223 in males and females	1) Coronary Heart Disease 2) Stroke	12	–	1) Fibrinogen in Males and Females 2) Fibrinogen in Males

* The number of subjects on which the published data analysis was based (not necessarily the total number eligible or entered).
† All subjects were age 54 at entry and were followed 13.5 years.
# This table does not include all endpoints for some studies.
Δ Only for events within 5 years of entry.
Ref. = reference
No. = number

identifying eligible subjects and the high rate of participation of eligible subjects makes it highly probable that a representative sample of Swedish males was studied.

By 13.5 years of follow-up, 92 men had experienced myocardial infarction, 37 had suffered a stroke, and 60 were dead from other causes. The entry values for cholesterol, systolic blood pressure, fibrinogen and smoking status were each significantly associated with subsequent myocardial infarction, while only the fibrinogen and systolic blood pressure were significant predictors of stroke (Table 7–3). When systolic blood pressure, cholesterol and smoking status were adjusted for in multivariate analysis, the association of fibrinogen level with infarction did not reach statistical significance ($p = 0.15$); while the association with stroke remained significant ($p = 0.05$). Other findings of the trial were a significant positive association of the P and P test, which measures the combined effect of factors II, VII, and X, with cholesterol but not with subsequent infarction or stroke. Fibrinogen was strongly correlated with smoking habit. Since this is a relatively small study, it is prone to Type II error, that is, the failure to detect a significant association due to insufficient statistical power. Therefore one cannot put too much weight on the failure to demonstrate that fibrinogen was an independent risk factor for infarction by multivariate analysis. Since apparently no exclusions were made at entry for pre-existing diseases under treatment, and some of the males in this age group can be presumed to have been under medical care, there is also the possibility of a confounding effect of treatment on the baseline variables measured.

This study illustrates the multiplicative effects of the presence of two or more risk factors, in this case for stroke. When subjects were grouped by low, intermediate or high levels of fibrinogen and blood pressure, those with low fibrinogen and systolic blood pressure had virtually no risk of stroke, while those with one high risk factor had a 1 to 4 percent risk of stroke, and those with both factors high had a 9-12 percent risk of stroke (Figure 7–2).

Table 7–3. The Gothenberg Study. Mean Values for Coagulation Factors and Conventional Risk Factors at Base-Line Examination, According to Endpoint

Variable	*No End-Point (n = 608)*	*Endpoint: Myocardial Infarction (n = 92)*	*Endpoint: Stroke (n = 37)*	*Endpoint: "Other Death"* (n = 60)*
Factor II-VII-X (%)	94.9	95.6	94.7	89.1
Factor VIII:C (%)	122	127	129	133
Plasminogen (U)	18.4	18.6	18.7	18.8
Fibrinogen (g/liter)	3.30	3.56†	3.70†	3.27
Fibrinolytic activity (mm^2)	135	132	134	133
Systolic pressure (mm Hg)	143	152‡	153§	145
Smoking (score)	2.6	2.9§	2.7	3.1‡
Serum cholesterol (mmol/liter)	6.95	7.44†	7.26	7.12

* Death unrelated to stroke or infarction.
† $P < 0.01$ (vs. no endpoint).
‡ $P < 0.001$ (vs. no endpoint).
§ $P < 0.05$ (vs. no endpoint).
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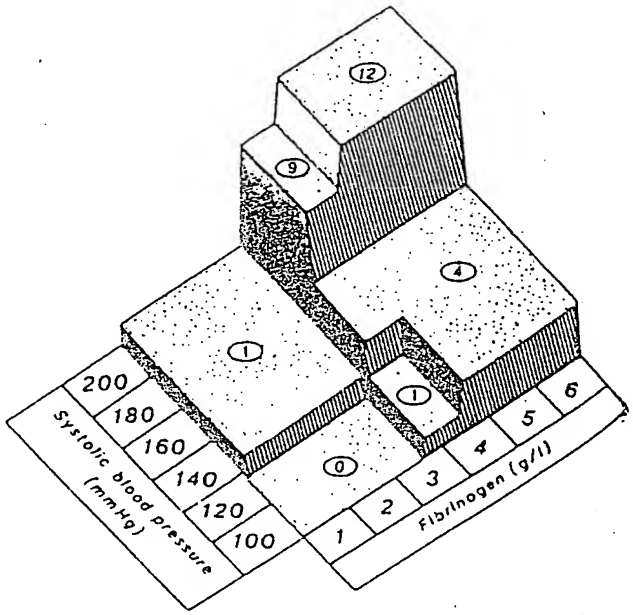

Incidence of Stroke (Circled Percentages), According to Groups Delineated by Various Combinations of Systolic Blood-Pressure Levels and Fibrinogen Levels. Reprinted by permission of the New England Journal of Medicine 311:504, 1984.

Figure 7-2. The Gothenberg study.

The Leigh Study

This epidemiologic study (94) prospectively assessed possible risk factors for the development of myocardial infarction in men drawn from a single general medical practice in Leigh, England (Table 7-2). Of 505 men between the ages 40–69 in this practice, 384 (76 percent) were examined at the beginning of the study, and after exclusions for pre-existing ischemic heart disease, diabetes, hyperlipidemia or hypertension under treatment, the results for the remaining 297 (59 percent) were analyzed. The socioeconomic characteristics of the group were not reported. Approximately 54 percent of the men were smokers. The baseline studies included an electrocardiogram, lipoprotein analysis, serum cholesterol, and plasma fibrinogen (by nephelometry of heat-treated plasma). These 297 men, whose average age at entry was 52.4 years, were followed an average of 7.3 years (range 0.1 to 16.1 years), with >50 percent followed at least 5 years (Table 7–2).

During this observation time, 40 men suffered myocardial infarctions, and were then compared to the 257 in the infarction-free group. The mean entry levels of plasma fibrinogen, systolic blood pressure and cholesterol were each highly significantly correlated with the occurrence of myocardial infarction by univariate analysis. Since the mean age of the 40 men with infarction was significantly older (58.4 years) than the attack-free group (50.6 years), the investigators divided the subjects into two groups, those 40–54 years-old, and those 55–69 years-old at entry. When fibrinogen levels were compared in these subgroups, fibrinogen level was still significantly correlated with risk of infarction. Multivariate analysis supported the likelihood that fibrinogen was an independent risk factor for myocardial

infarction. This was a small study from a single medical practice, and it is possible that the results are not generalizable to an unselected larger population. However the findings agree closely with those from the larger Northwick Park and Framingham studies, which will be discussed later in this review.

Those subjects whose systolic blood pressure was in the top third and who also had a fibrinogen level in the top third had a 12-fold increased risk of myocardial infarction during the observation period compared to those in the lowest third for fibrinogen. A similar but less striking combined effect of high cholesterol and high fibrinogen on risk of myocardial infarction was also found. These findings are analogous to the combined effect of systolic blood pressure and fibrinogen on risk of stroke in the Gothenberg study. The possible underlying physiologic explanations for such a multiplicative effect include the hypothesis that increased systolic blood pressure coupled with increased plasma fibrinogen greatly increases the incorporation of fibrinogen into arteriosclerotic plaque formation at the site of intimal damage.

The Northwick Part Heart Study (NPHS)

This prospective epidemiologic study was begun in 1972 to evaluate the possible relation of various hemostatic parameters, which might promote thrombosis, to the development of ischemic heart disease (9,62). Subjects were recruited primarily from the London headquarters and production site of a food-processing company (H.J. Heinz Co.) by individual invitation to all employees who had worked for the company at least 18 months; the participation rate of this group was reported as approximately 80 percent. Other subjects were also recruited by general invitation from a group of civil servants and postal workers; for this group the number of subjects eligible and the participation rate were not accurately known. Males and females were eligible, and the age ranges were 18 to 64 years for males and 18–59 years for females in the first group of subjects recruited (1426 men and 638 women) (62). The mean age of the men was 43.9 years, with 39 percent under the age of 40, and the mean for women was 43.9 years, with 33 percent under the age of 40. Approximately 90 percent of the participants were white. Forty-three percent of the men were current smokers. Social class distribution was similar in males and females, with about half in the mid-level social class III (skilled trades and low level white-collar positions) and about one-fourth each in the higher and lower groups. Thus the enrollment of subjects appears to have a broad representation of social class. Follow-up was planned for at least 6 years from entry. Subjects were not excluded because of pre-existing disease; 7 percent of the males age 40 or older had been hypertensive and many of these were on treatment, and 60 males had histories of angina or previous myocardial infarction.

Laboratory parameters studied at entry included: factor V and factor VII (the latter by a one-stage clotting assay in human factor VII-deficient plasma), factor VIII by a two-stage clotting assay, fibrinogen by a gravimetric technique (29), fibrinolytic activity by dilute blood clot lysis time, antithrombin III by biologic and immunologic methods, and platelet count and adhesiveness. Blood samples were obtained from day workers in the morning in the non-fasting state and also on the next day in the fasting state, and from night workers in the morning in the non-

fasting state. Other data collected included blood pressure, electrocardiography, smoking and alcohol consumption, blood lipids, and measures of obesity. Since entry assays for hemostatic variables were performed over a time span of 6 years, considerable attention was paid to standardization of the assays and control of temporal drift in the assays.

The first report of results from this study (64) analyzed the results as of 1979 for 1511 white men age 40–64 years at recruitment, out of a total study group of nearly 3500 men and women. An independent panel of physicians reviewed the clinical charts and autopsy reports of all deaths to determine the classification of cause of death as cardiovascular or other. Statistical analysis for significance was performed by comparison of the geometric means of the group who died from cardiovascular disease (N = 27) and the survivors without ischemic heart disease (N = 1461) for each of the 10 quantitative variables measured at entry (8 hemostatic variables, serum cholesterol, and systolic blood pressure). Significant correlations were found between each of three hemostatic variables—factor VII, factor VIII, and fibrinogen—and subsequent cardiovascular death, with p values of <0.01 for factor VII and fibrinogen, and < 0.05 for factor VIII.

A subsequent report from the NPHS was based on a longer follow-up of the same subgroup—1511 white men between the ages of 40–64 at entry (66) (Table 7–2). Analysis of mortality was based on a mean follow-up of 10.0 years (range 7.3–13.5), with a mean interval from entry to death from ischemic heart disease of 5.9 years. Non-fatal events were determined during a clinical re-examination, which took place a mean of 6.7 years after entry (range 2.1–12.6 years), with a mean interval from entry to non-fatal myocardial infarction of 4.3 years. Thus the total follow-up period for fatal events was longer than for non-fatal events. A total of 60 non-fatal myocardial infarctions were documented from questionnaires sent to participants and their primary physicians 3 years after entry and from the clinical re-examination. A total of 143 deaths occurred during follow-up, 99 of them before re-examination was to take place. The independent panel of physicians determined that 68 of these 143 deaths were from ischemic heart disease, with 28 of them sudden deaths. Autopsies were performed on 71 percent of the subjects whose deaths were judged to be from ischemic heart disease. The 52 subjects with a history of myocardial infarction before entry into the study were excluded from the analysis of non-fatal myocardial infarction but not from the analysis of deaths from ischemic heart disease. By multivariate analysis, the factor VII level measured at entry was significantly associated with death from ischemic heart disease within five years of entry and for the total follow-up period (Table 7–4). Fibrinogen level at entry was also significantly associated with subsequent death from ischemic heart disease (Table 7–4). For non-fatal myocardial infarction, both the fibrinogen and factor VII levels were significantly associated with subsequent events within five years of entry, but only the fibrinogen level was significantly associated with events for the total follow-up period (Table 7–4). In addition, when the 52 subjects with previous myocardial infarction were excluded, the association of fibrinogen with death from ischemic heart disease was still significant ($p = 0.03$). The actual number of events, whether fatal or non-fatal, was 2–3 times greater in the highest third for factor VII (above 120 percent) or fibrinogen (above 3.2 grams per liter) than in the lowest third for each variable. Factor VIII level at entry was not significantly

Table 7–4. Selected Results of Northwick Park Heart Study

	Factor VII (%)		*Fibrinogen (g/L)*		*Cholesterol (mmol/L)*	
	≤5 yr	*Total*	*≤5 yr*	*Total*	*≤5 yr*	*Total*
I.H.D. Deaths (N = 68)						
$\bar{x}$ value	123.9	117.2	3.25	3.13	6.59	6.43
p value*	0.002	0.002	0.005	0.002	0.01	0.003
Non-Fatal M.I. (N = 60)						
$\bar{x}$ value	113.9	107.3	3.29	3.22	6.26	6.14
p value*	0.03	N.S.	0.0009	0.001	N.S.	N.S.
Survivors without M.I. (N = 1280)						
$\bar{x}$ value		107.0		2.90		6.00

* For regression coefficients from multivariate analysis
$\bar{x}$ = mean
yr = year
N.S. = not significant
I.H.D. = ischemic heart disease
M.I. = myocardial infarction

associated with either fatal or non-fatal events for the total follow-up period. The serum cholesterol level at entry was significantly associated with deaths from ischemic heart disease but not with non-fatal myocardial infarction (Table 7–4).

The Framingham Study

Beginning in 1948, the Framingham study has biennially followed a cohort of 5209 male and female residents of the town of Framingham, Massachusetts, by history, physical examination, and testing for a number of variables as possible risk factors for arteriosclerotic vascular disease. The endpoints in this study are: first attack of coronary heart disease, stroke, cardiac failure, or occlusive peripheral vascular disease. An initial sample of 6507 men and women were individually invited to attend the study clinic for examination, and 4494 (68.8 percent) actually did so (36). This participation rate was considered disappointing, and the initial sample was increased by addition of some volunteer residents who had not been part of the original sample list. There was some indication from mortality data that the respondents from the initial sample were healthier than those who refused to participate, and that women who failed to return after the initial visit were less healthy than those who did. The possibility that the sample was not completely representative of a general population was considered in the design of the data analysis.

Some of the variables initially measured as possible risk factors included blood pressure, relative weight (as an index of obesity), serum cholesterol, VLDL (see below), uric acid, and glucose. After the midpoint of the tenth exam in 1968, a fibrinogen level was added to the battery of laboratory tests. The fibrinogen assay was a modified version of the Ratnoff and Menzie method, which involves clotting

Table 7–5. Risk of Coronary Heart Disease by Fibrinogen Level at Examination 10, Framingham Study*

Tertile of Fibrinogen, g/L (mg/dL)	Total No. of Events		12-y Age-Adjusted Rate of Coronary Heart Disease per 1000			
			Age 47–79 y		Age 47–59 y†	
	Men	Women	Men	Women	Men	Women
1.3–2.7 (126–264)	35	20	178	94	125	70
2.7–3.1 (265–311)	35	23	176	94	170	89
3.1–7.0 (312–696)	41	48	287‡	169‡	358§	160‡

* The population at risk consists of subjects who are free of cardiovascular disease at examination 10.

† Trends significant in both men and women ($P < .05$).

‡ $P < .05$.

§ $P < .01$.

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plasma and then removing, drying, and hydrolyzing the clot, before measuring the final product spectrophotometrically. Results were obtained in 1499 subjects—662 men and 837 women (53). Subjects who had already experienced a cardiovascular event were excluded from the analysis; thus 554 men and 761 women followed for 12 years formed the basis of the analysis (Table 7–2). The age range was 47 to 79 years, with a mean above 55 (exact value not stated), making this a somewhat older cohort at entry than the Northwick Park, Gothenberg, or Leigh studies.

During the 12-year follow-up, 312 cardiovascular events occurred, including 214 first episodes of coronary heart disease (117 in men and 97 in women) and 92 strokes. The mean fibrinogen level at entry was 285 mg/dL in men and 296 mg/dL in women, which was not significantly different. Risk of coronary heart disease was significantly greater in the upper third of fibrinogen values (>312 mg/dL) for both men and women (Table 7–5). Risk of stroke was also positively related to the fibrinogen level in men. Fibrinogen was positively correlated with a number of other risk factors, including smoking, as had been demonstrated by the Gothenberg and Northwick Park studies. Despite these correlations, multivariate analysis supported the conclusion that fibrinogen was an independent risk factor for overall cardiovascular disease (combining all 4 endpoints), and for coronary heart disease in men ($p < 0.01$), and for coronary heart disease in women ($p < 0.005$); the correlation with overall cardiovascular disease in women was of borderline significance. Whereas the fibrinogen level was significantly correlated with risk of coronary artery disease in men and women, for stroke the association was significant only for men (54). For women, the effect of fibrinogen on overall risk of cardiovascular disease declined with age and had no apparent impact over the age of 70 (Fig. 7–3). This is similar to the declining importance of serum cholesterol as a risk factor in older women in the Framingham study and emphasizes the importance of considering the age of the study group when extrapolating results of an epidemiologic study to a general population or a clinical practice.

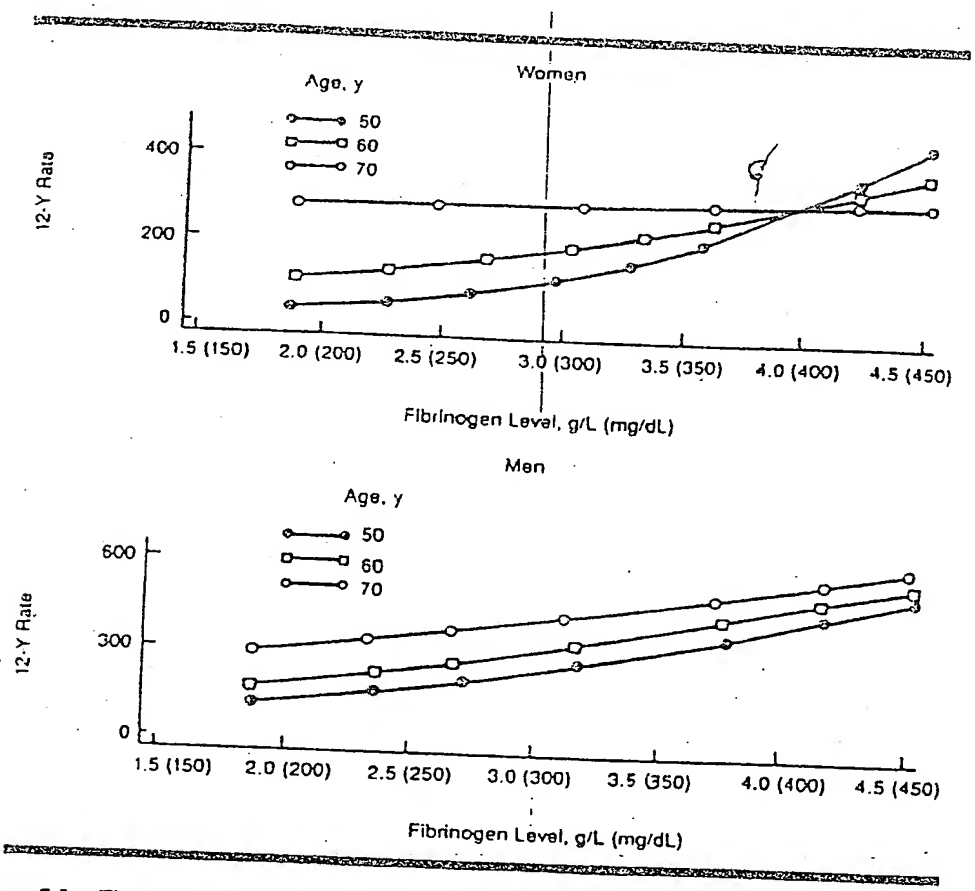

Figure 7-3. The Framingham study. Risk of cardiovascular disease by fibrinogen level and age, 12-year follow-up (examinations 10 through 12). Reprinted by permission of the Journal of the American Medical Association, Vol 258, p. 1184, 1987. copyright 1987, American Medical Association.

The Münster Study

Another large prospective epidemiologic study of risk factors for arteriosclerotic disease is in progress in Münster, Germany and is known as the PROCAM study (an abbreviation for Prospective Cardiovascular Münster). Begun in 1979, this study enrolled more than 11,000 men and 5,000 women by 1986 (1). The design is similar to the Framingham study, with an initial examination followed by biennial monitoring by questionnaire and re-examination every 4–5 years (3). The eligible population consisted of employees of companies and civil servants in the geographic district of Westphalia, and about 60 percent volunteered to participate. The use of employees is similar to the eligible population from which the Northwick Park study drew its participants. Since both of these studies did not include unemployed

or retired workers or homemakers, it is possible that these study groups are only partly representative of the more general population, especially in the case of women.

Variables measured at entry included blood pressure, weight, height, electrocardiogram, smoking habit, alcohol consumption, family history and individual history; 20 laboratory tests were also performed, including fasting blood lipids, glucose, and uric acid. Based on the preliminary data from the Northwick Park study, in 1981 the coagulation parameters fibrinogen, factor VII and factor VIII were added. Fibronogen was measured by the method of Clauss (a modification of the thrombin clotting time), and factor VII and factor VIII by one-stage clotting assays. Occurrence and confirmation of cardiovascular events was monitored by questionnaire, by re-examination, and by contacting families, physicians, and hospitals. Endpoints included myocardial infarction, stroke, and death from atherosclerotic disease.

A report of the baseline characteristics of 4186 subjects (2880 men and 1306 women) showed that factor VII, factor VIII and fibrinogen each increased with age over the age range of 20–59 (3). In men this increase was roughly linear, while in women there was little rise of factor VII or fibrinogen before age 40 and then a relatively steep rise, compared to men, from age 40–59. Factor VII level was also positively correlated with oral contraceptive use, menopausal status, and body weight; fibrinogen was also positively correlated with cigarette smoking (both the fact of being a smoker and the number of cigarettes smoked per day), oral contraceptive use, and body weight. A further report of the baseline characteristics of the PROCAM study subjects included multivariate analysis of the relation of each of the clotting factors to blood pressure, blood glucose, uric acid and lipid fractions (total serum cholesterol, HDL- and LDL-cholesterol, and triglycerides) (4). This analysis showed that there was a highly significant correlation of the factor VII level with blood glucose and with triglycerides in men and with HDL-cholesterol and triglycerides in women. Fibrinogen was strongly linked to LDL-cholesterol and to factor VIII in men, but in women it was only linked to factor VIII. A similar association of the clotting factors with lipid fractions had been suggested in the Northwick Park and Framingham studies, but not in as great detail.

Results from the PROCAM study indicate that hypertension, diabetes mellitus, and hyperlipidemia were all independent risk factors for coronary heart disease in men (2). Hyperlipidemia appeared to be the most important of these risk factors, and the presence of two risk factors had a multiplicative effect on the incidence rate of coronary heart disease. This analysis was based on data from 2754 men who had no history of myocardial infarction or stroke at entry and who were followed for four years, during which 73 suffered coronary events. No full report of a similar analysis for clotting factors has appeared yet, since these parameters were only included in the study as of 1981. However, a preliminary report was published, based on the observation of 1674 men (mean age 48.4 years at entry) followed for at least 2 years, during which 15 coronary events were recorded. Compared to the 1659 men with no event recorded, these 15 subjects had higher mean factor VII, fibrinogen, total and LDL-cholesterol levels and blood pressure, and lower mean HDL-cholesterol levels (5). Since 14,799 men and 6507 women were entered in the PROCAM study, one would expect that longer follow-up of this large cohort could yield important data to help clarify the relative importance

of the clotting factors, and factor VII in particular, as risk factors for coronary heart disease.

GENERAL CONSIDERATIONS OF DESIGN AND INTERPRETATION OF PROSPECTIVE EPIDEMIOLOGIC STUDIES

Sample Size

Phillips and Pocock (82) recently reviewed the statistical estimation of required sample size in designing a prospective study with the aim of testing whether a putative risk factor is associated with disease by univariate analysis and whether the association is still significant after adjusting for other known risk factors in multivariate analysis. Using coronary heart disease as their example, they demonstrated that one key issue of study size is the number of cases of the disease required, which will depend on the incidence rate in the population under study and the period of observation. For a risk factor with a strong association on the order of cholesterol or blood pressure, univariate analysis may require as few as 50 cases, while for weaker risk factors as many as 200–400 cases may be needed. They estimated that for multivariate analysis to prove independence of one risk factor from all other known risk factors, about 20 to 50 percent more cases are needed than for univariate analysis. Even larger number of cases will be required when the factor being tested is highly correlated with other known risk factors. Thus, estimation of adequate study size is based on a number of assumptions that, in a given case, may be erroneous. Nevertheless, on the basis of this kind of estimation of study size, one may hazard a guess whether a particular study was large enough for the purpose of testing a possible new risk factor. A major precaution to be gleaned from this kind of analysis is that any study that fails to prove the importance of a particular risk factor may simply not have had the statistical power to do so.

All four prospective studies of fibrinogen as a risk factor found a similar, strong predictive power of fibrinogen level at entry for subsequent coronary heart disease. These studies together evaluated a combined total of more than 400 men with myocardial infarction or sudden death and many thousands of men without such events. Thus, the association of plasma fibrinogen level with coronary heart disease in men appears to have been established beyond any reasonable doubt. It is also obvious, by contrast, that association of factor VII with coronary heart disease is not based on nearly the same sample size and thus requires confirmation and further study. More data are also needed for evaluation of these risk factors in women.

Sample Composition

Most epidemiologic studies are drawn from a restricted geographic area, and it is possible that some of the results obtained are not applicable to people of different genetic, occupational, or social backgrounds. Despite the investigators' attempts to have a random sample, some selection bias may occur. These limitations

mean that in practice single studies rarely can reliably prove the importance of a given variable as a risk factor for developing a specific disease or condition in the public at-large, and similarly a second study in a different population sample cannot disprove the first study.

The Triglyceride Controversy

An example of the controversy that can arise from conflicting results of statistical analysis by multiple regression (multivariate) methods in different studies is the case of serum triglycerides, which was found to be a risk factor for coronary heart disease in men in some studies but not in others (38). The Stockholm prospective study found the fasting triglyceride level to be a significant risk factor in men after taking into account the correlation of triglycerides with other risk factors, including cholesterol (11). Similar findings were reported from Finland (80). In contrast, the Framingham study did not find an independent association of the prebeta S_f 20-400 lipoprotein level (drawn in a random non-fasting state) with risk in men; in women, this very low density lipoprotein fraction (VLDL), which is composed mainly of triglyceride, was a significant risk factor and appeared to be more important than the total serum cholesterol in women over the age of 50 (52). Both the high variance in triglyceride measurements, particularly non-fasting, and the strong correlation with cholesterol levels may have diminished the ability of multivariate analysis to demonstrate a separate contribution of triglycerides to risk of cardiovascular disease in men. One physiologic reason for the statistical linkage of these two lipids as risk factors may be related to the demonstration in a Seattle study that combined familial hyperlipidemia; in which cholesterol or triglycerides or both are modestly elevated in heterozygous affected family members, is the most common genetic category of hyperlipidemia in survivors of myocardial infarction under the age of 60 (34). In a 1980 review, Hulley and coworkers illustrated the use of logistic regression analysis in assessing the importance of fasting triglycerides in the Western Collaborative Group Study of men aged 41–61 at entry. They concluded that the triglyceride level was not a significant risk factor when HDL-cholesterol and body mass index were taken into account (49). However, a re-analysis of the data from the framingham study led to the conclusion that the serum triglyceride level was, after all, a risk factor in men over 50 years old (17). This controversy is pertinent to the issue of coagulation factors as possible risk factors both as an example of the limitations of multivariate analysis and because of the linkage of factor VII and triglyceride levels.

CROSS-SECTIONAL STUDIES OF FIBRINOGEN AND ISCHEMIC HEART DISEASE

MacDonald and Edgill in 1957 (61) reported a study from London that found higher mean fibrinogen levels in subjects with coronary artery disease than in normal controls. A few years later, Merskey and coworkers published a report from South Africa in which they compared a number of possible risk factors for

coronary artery disease in Bantu males (a group with a very low incidence of coronary artery disease), white males with coronary artery disease, and age-matched healthy white males (69). Fibrinogen was measured by the gravimetric technique. They found that the mean fibrinogen level of men with coronary disease was significantly higher than that of age-matched white male controls. Since previous investigators had found transient elevations of fibrinogen for up to three months after acute myocardial infarction (33), Merskey and coworkers were careful to evaluate this possible explanation of their results but found no difference between their subjects with more recent versus remote history of infarction. No other statistically significant differences were found, but the Bantus had a lower factor VII activity than either group of white males, a lower prothrombin level, and much lower cholesterol; the latter was possibly related to the Bantu diet, which typically contained only 18 percent fat. More recently, investigators have reported higher fibrinogen levels in patients with two- or three-vessel coronary artery disease compared to a single vessel or no disease (57) and in patients admitted for unstable angina, compared to controls (95). In another study, higher plasma viscosity was found in patients with unstable angina and acute myocardial infarction, but not stable angina, compared to controls (32).

There is little evidence that fibrinogen level is linked to dietary fat intake. Dietary modification (reduction in saturated fat or increase in fiber) has not been shown to lower fibrinogen significantly (25,30), nor did a high fat diet raise fibrinogen (72). No significant difference in fibrinogen levels was found in white British vegetarians and non-vegetarians (41). On the other hand, rural South African black males on a high-egg diet (high cholesterol but low fat) had significantly higher fibrinogen levels than a group of comparable males on the usual low fat diet (98). A group of patients with Type II hyperlipidemia (some of whom had hypertriglyceridemia) were found to have higher fibrinogen levels than normal controls (56). Another study, which included subjects with hyperlipidemia types IIa, IIb, IV, and V, found elevated mean fibrinogen levels in types IIa and IIb compared to normal but the differences were not statistically significant (20).

These studies utilized a case-comparison design in which a group of cases considered to have a disease, to be at high risk of the disease, or to have certain characteristics (by some pre-established criteria) are compared to another group of subjects at lower risk by these criteria or with a different set of characteristics. None of the groups in such cross-sectional studies is likely to have been selected by random sampling techniques from a much larger group of eligible participants. Rather, they are usually the subjects, meeting the study criteria, who are readily available to the investigators. Conclusions from such cross-sectional studies should be considered tentative because of the uncertainty as to whether the two groups are truly comparable for all other variables (besides the one being tested) that might affect fibrinogen level. Since fibrinogen is an acute-phase reactant (as is factor VIII), it may be elevated by a number of inflammatory conditions unrelated to ischemic vascular disease. For instance, rural black Gambians (67) and Bantus (69) had high fibrinogen levels compared to whites, despite the fact that these rural black groups had very low rates of coronary artery disease. Meade (67) postulated that the presence of endemic parasitic disease caused the higher fibrinogen levels in the rural blacks and that one or more other risk factors (e.g. smoking or hy-

pertension) must co-exist with the elevated fibrinogen to increase the risk of arteriosclerosis (67).

As discussed above, fibrinogen level is positively correlated with cigarette smoking and with body weight. Recent reports also link fibrinogen level with social class, being higher with lower social class, even after the contribution of smoking is taken into account (59). This may partly explain the higher risk of ischemic heart disease found in the lower grades of employment in the Whitehall study in London (60). There is evidence linking job stress with fibrinogen level (59) and exam stress with increased plasma viscosity (26), supporting a hypothesis that chronic adrenergic stimulation may increase fibrinogen level. Genetic influences may also control the plasma fibrinogen level. A study of 91 unrelated individuals found evidence linking fibrinogen level to phenotype for certain restriction fragment length polymorphisms of the fibrinogen genes and suggested that this may account for approximately 15 percent of the variance in fibrinogen between individuals (50). Other investigators, who studies families in Sweden, concluded that about 50 percent of the variance in fibrinogen was genetically determined (42). These preliminary studies are intriguing and suggest the possible importance of fibrinogen in explaining the familial clustering of ischemic heart disease, which cannot be fully explained by other risk factors.

The pathophysiologic mechanisms by which the plasma fibrinogen concentration may contribute to the etiology and progression of ischemic cardiovascular disease were mentioned in the introduction. In addition to the influence of fibrinogen level on plasma viscosity and the role of fibrinogen in platelet aggregation, some studies show a positive effect of fibrinogen concentration on incorporation of fibrin into atheromatous plaque (58). The evidence for the various possible mechanisms of fibrinogen's role in atherosclerosis has been well reviewed recently (93). There is surprisingly little experimental evidence to support the hypothesis that increasing the fibrinogen concentration in the physiologic range increases the amount or rate of clot formation in vitro or in vivo. One study in endotoxin-induced intravascular coagulation in rabbits showed that fibrinogen concentration increased the amount of intravascular fibrin deposition, compared to control animals, but not in animals made leukopenic by nitrogen mustard (39). Deduction from this model to the situation of thrombosis occurring at an arteriosclerotic plaque is, of course, problematic.

IMPORTANCE OF ASSAY METHODS FOR FIBRINOGEN AND FACTOR VII

The four prospective studies that showed fibrinogen level to be a risk factor for ischemic heart disease used different assay methods but all found quite similar results, with the greatest risk of disease occurring in subjects whose fibrinogen was >3.0 grams per liter approximately. Thus it would appear that the assay method was not critical. The agreement between these large studies probably reflects the careful attention paid to standardization of each assay method. In order to interpret laboratory results on individual patients not part of such a well-controlled study, it would be very helpful for laboratories to have available a national or international

reference standard to use for calibrating their in-house standards. Such a reference standard has recently become available from the College of American Pathologists in the U.S. and another is in preparation in London.

The assay method most widely used for assaying factor VII activity has been the one-stage modified prothrombin time, in which test plasma is added to plasma from a patient congenitally deficient in factor VII, and a mixture of thromboplastin and calcium are then added. Most commonly the source of tissue factor is rabbit or human, and there does not seem to be a major difference between assays using these two species. Bovine tissue factor reacts well only with activated human factor VII and not the zymogen factor VII (97). Therefore assays with bovine thromboplastin can reflect activation of factor VII in test samples. Another potential problem in assaying factor VII is the phenomenon of cold-activation of factor VII, which occurs in plasma (held at <4°C, especially in glass) from pregnant women, from a small percentage of otherwise healthy individuals, and from some patients with coronary artery disease (35).

Several methods have been developed to detect the presence and estimate the level of activated factor VII in test samples. One of these methods depends on the different sensitivity of bovine tissue factor for factor VIIa versus factor VII (97). Two other methods—the coupled amidolytic assay (88) and the tritiated peptide release assay (71)—utilize the comparison of coagulant activity with a measurement of factor VII mass. The estimation of total factor VII mass depends on the conversion of factor VII to VIIa in the test sample with subsequent measurement of the amount of factor X activation. Since these are activity assays, it is possible that under some circumstances these assays may be affected by other variables in addition to the level of factor VII, such as the level of extrinsic pathway inhibitor. For these reasons, it is likely that an antigen assay for factor VII is a superior method for estimating factor VII mass. An enzyme immunoassay for factor VII antigen has been found to be highly specific and reproducible (96) and has been used in studies of ischemic heart disease and hyperlipidemia (see below).

CROSS-SECTIONAL STUDIES OF FACTOR VII AND ISCHEMIC HEART DISEASE

Dalaker and coworkers (22) studied factor VII levels in thirty-six 42 year-old male subjects, drawn from the Oslo Age 40 study, who had been characterized as being either low risk or high risk for coronary artery disease by predetermined criteria. The latter group was subdivided into an intervention group and a non-intervention group. They found that the high risk group ($n = 25$) had a mean factor VII level of 117 percent, which was significantly higher than the low risk group ($n = 11$), whose plasma pool was defined as 100 percent. These investigators also found that factor VII in the high risk plasmas was decreased to a greater extent by phospholipase C addition than factor VII in the low risk plasmas. The level of this phospholipase C-sensitive factor VII fraction (but not total factor VII) was significantly correlated with the fasting serum triglyceride level, but not with cholesterol. Interestingly, the high risk intervention group (studied after dietary intervention) had a 25 percent lower level of cholesterol than did the high risk non-

intervention group and yet had no difference in factor VII levels. The addition of diisopropylfluorophosphate (a serine protease inhibitor) to high risk plasma appeared to inactivate somewhat more factor VII than an equivalent addition to low risk plasma. Dalaker and coworkers postulated the existence of a plasma factor VII-phospholipid complex with greater enzymatic activity than uncomplexed plasma factor VII. This explanation was also proposed for similar findings on plasma of pregnant women, who have very high levels of factor VII by the third trimester (21). This group is not at high risk of coronary artery disease but does have hypertriglyceridemia. In a subsequent publication, Dalaker and coworkers (23) found that the phospholipase C-sensitive fraction was 29 percent (mean) of total factor VII activity in 100 male survivors of myocardial infarction compared to 7 percent in 48 healthy control men ($p < 0.001$). This fraction was again highly correlated with triglyceride level but not with cholesterol, and not with HDL-cholesterol if adjustment for triglyceride correlation was first made. The factor VII activity assay in all the studies by Dalaker and coworkers was a one-stage assay performed with human thromboplastin and plasma from hereditary human factor VII deficiency.

In a study at Stony Brook, which compared middle-aged subjects (mean age 52 years) prior to elective coronary arteriography to healthy low-risk age-matched subjects, we found a significantly higher mean factor VII activity (116 percent) in the former compared to the latter (97 percent) using a one-stage clotting assay with human factor VII-deficient plasma and rabbit tissue factor (46). Using the coupled amidolytic method to assess the presence of activated factor VII (88), we found no evidence to support an increase in activated factor VII as the explanation for the increase in factor VII activity. The elevated factor VII activity in the subjects undergoing arteriography was significantly associated with fasting serum triglyceride levels, but not with cholesterol. In a subsequent study, we compared factor VII activity and antigen levels in young asymptomatic first-degree relatives of patients with premature coronary artery disease and low-risk age-matched subjects (47). The former group had significantly elevated mean factor VII activity (116 percent) and antigen (125 percent) compared to low-risk subjects (103 percent and 97 percent respectively), and there was no evidence for an increase in activated factor VII by comparison of the coagulant activity with either antigen levels, measured by enzyme immunoassay, or amidolytic activity, measured by the Seligsohn method.

Other investigators have used functional and immunologic factor VII assays to assess the activity state of factor VII in patients with unstable angina and acute myocardial infarction (13). Factor VII activity was elevated much more than antigen in both groups, suggesting the presence of activated factor VII, and was correlated with the level of thrombin-antithrombin complexes. In contrast, another group found no significant increase in factor VII coagulant or amidolytic activity in fasting specimens from patients 1–4 days after acute myocardial infarction, although patients hospitalized for chest pain who did not develop infarction had mildly elevated factor VII levels by both assays (86). These investigators also found significantly elevated extrinsic pathway inhibitor levels in patients with myocardial infarction and severe angina without infarction and an elevated phospholipase C-sensitive factor VII fraction in acute myocardial infarction.

STUDIES OF THE RELATION OF FACTOR VII AND SERUM LIPID LEVELS

Constantino and coworkers (20) measured a large number of coagulation factors (II, V, VII, VIII, IX, X, and fibrinogen) in a group of patients with well-characterized hyperlipidemias (types IIa, IIb, IV, and V). They found elevated levels of factors II, VII, IX and X in all 4 types of hyperlipidemia; these differences were statistically significant for factor II in type IIa, factors II, VII, IX, and X in type IIb, and factors II, VII, and IX in type V. Studies with plasma lipoprotein fractions prepared by density gradient ultracentrifugation found that factor VII and X bound to chylomicra, VLDL, and LDL and that treatment of the lipoprotein fraction with phospholipase C increased the binding (14). This observation suggests a possible explanation for the findings of Dalaker and coworkers that phospholipase C addition decreased the measurable factor VII activity in some patients' plasma more than in normals. Perhaps the phospholipase C increased the binding of factor VII to triglyceride-rich lipoproteins in those patients' plasma and thereby might have interfered with its coagulant activity. In a recent study of 145 middle-aged men in whom lipoprotein fractions were characterized and factor VII activity measured, factor VII level correlated best with the large triglyceride-rich fractions (chylomicra and VLDL) (76).

The involvement of carbohydrate metabolism in the control of factor VII levels was suggested by a study which measured factor VII activity and glucose levels in the fasting state and after an intravenous glucose load in both normal subjects and patients with insulin-dependent diabetes (18). Factor VII levels correlated significantly with fasting glucose levels in both groups, and factor VII rose significantly during the induced hyperglycemia in both groups. Dietary studies have also linked elevation of the vitamin K-dependent factors with hypertriglyceridemia. In contrast to the lack of effect of diet on fibrinogen levels, patients with marked hypertriglyceridemia (5–6 mmol/L) have shown significant reductions of factor VII and X after treatment with diet alone or diet and clofibrate (25; 92). Serum triglycerides were reduced >50 percent after therapy in both studies. The study by Elkeles and coworkers also measured prothrombin levels, which were elevated before therapy (mean of 144 percent) but only decreased modestly after therapy (mean of 133 percent). When normal subjects were studied on low versus high fat diets, factor VII activity correlated positively with fat and protein intake, and as little as one day of high fat intake raised the factor VII coagulant activity (72).

Animal studies have also addressed the relation of diet to elevation of vitamin K-dependent coagulation factors. Rabbits fed a 1 percent cholesterol-supplemented diet had a 20-fold increase in plasma cholesterol (predominantly in VLDL) and a 3-fold increase in factor VII coagulant activity (75). Total factor VII (measured by tritiated peptide release assay on serum) also rose, but only about 1.3 fold, so that the ratio of coagulant activity to total activity increased in the cholesterol-fed rabbits. This observation suggested that an increase in activated factor VII largely explained the marked increase in factor VII coagulant activity. Prothrombin and factor X in these rabbits were also elevated when measured by amidolytic assays but not by coagulant assays. In a related study, Mitropoulos and Esnouf (74) measured the turnover of prothrombin and factor X in cholesterol-supplemented

rabbits and rabbits on a normal diet, using radiolabelled rabbit factor X and bovine prothrombin. The half-lives and fractional pool sizes for each protein were similar in the two groups of rabbits, as was the fractional catabolic rate. The absolute catabolic rate was significantly higher for each protein in the rabbits on a cholesterol-supplemented diet, leading the investigators to conclude that the synthesis of prothrombin and factor X was increased in this group.

Various studies have attempted to determine whether activation of plasma factor VII, rather than an increase in mass, explains the relation of factor VII activity with increased triglycerides. In a study of normal adults and pregnant women, Miller and coworkers (71) found that factor VII coagulant activity correlated significantly with both cholesterol and triglycerides, while total factor VII activity (measured as tritiated peptide release in serum) did not. Addition of LDL or VLDL lipoproteins to plasma did not alter the level of factor VII activity measured, ruling out an in vitro effect on the assay. The mean level of total factor VII (104 percent) was slightly higher than the coagulant activity (90 percent) in the normal adults, while the pregnant women had mean coagulant and total activities of 154 percent and 102 percent respectively. The increased ratio of factor VII coagulant activity to total factor VII in the pregnant women supported the conclusion that this group had an increase in activated factor VII. The authors hypothesized that factor VII activation explained the stronger correlation of factor VII clotting activity, compared to total factor VII, with triglyceride level in the healthy middle-aged adults.

In a study using an enzyme immunoassay for factor VII antigen, a similar and significant elevation of both factor VII activity and antigen was found in hypertriglyceridemia (type IV hyperlipidemia); the investivators concluded that total factor VII concentration was elevated in these patients (15). Patients with Type IIa and IIb hyperlipidemia had factor VII levels intermediate between those of the patients with Type IV and the healthy controls. The level of factor VII coagulant activity for the total group of patients was weakly correlated with the D-D dimer level. In a related study of 36 mildly hyperlipidemic patients given a standardized high-fat, high carbohydrate meal, those patients with elevated triglycerides (>150 mg/dL) 8–12 hr postprandially had significantly elevated factor VII activity and antigen compared to the patients with triglycerides <150 mg/dL (16). These investigators considered this additional evidence that hypertriglyceridemia was associated with an elevation in the total concentration of factor VII. Another study in Type IIa hyperlipidemia (cholesterol elevation alone) found both factor VII activity and antigen elevated, but only the former was statistically significant (10). Despite the fact that these were patients with hypercholesterolemia, the factor VII elevation by both assays correlated with the triglyceride level, not with serum cholesterol. One explanation for this, and a problem for interpreting all these studies of hyperlipidemia, is the observation that patients from families with combined hyperlipidemia can have a lipoprotein analysis consistent with either Type II (a or b), Type IV or Type V hyperlipidemia (44). In those patients with elevation of cholesterol or triglycerides alone, the diagnosis of combined hyperlipidemia can only be made with certainty by a family study. Thus the relative proportion of patients from families with combined hyperlipidemia compared to familial hypercholesterolemia in a study sample may affect the results obtained.

POSSIBLE IMPORTANCE OF LIPOPROTEIN COMPOSITION

The strong correlation of factor VII levels with serum triglyceride level raises the issue of the importance of lipoprotein composition and metabolism in atherogenesis, a subject which has been addressed elsewhere (79, 84, 99). There is a strong negative correlation between the serum triglyceride level and HDL-cholesterol, and the level of the latter is strongly negatively correlated with risk of ischemic heart disease (70, 90). The triglyceride-rich lipoproteins have been found to influence the particle size, composition and concentration of the HDL subfractions HDL_2 and HDL_3 (48). Several groups of investigators have shown that the composition of VLDL in hypertriglyceridemic patients differed from normal and might be involved in atherogenesis (24). The levels of apoproteins A-I and B (the principal apoproteins in HDL and LDL/VLDL respectively) are also significant correlates of risk of ischemic heart disease (31). An intriguing connection between coagulation and the apoproteins was provided by studies which showed that the apoprotein A-II component of HDL inhibited the activation of factor X by factor VIIa and tissue factor in phospholipid micelles (12). However, apoprotein A-II was not able to inhibit tissue factor expressed on cultured fibroblasts, an observation that creates doubt as to the biologic significance of this inhibition (37).

ROLE OF TISSUE FACTOR AND FACTOR VII AT THE SITE OF VASCULAR ENDOTHELIAL DAMAGE: POSSIBLE INVOLVEMENT IN THROMBOSIS AND ATHEROGENESIS

Tissue factor can be expressed on the surface of stimulated endothelial cells and in an experimental flow model it interacts with factor VIIa in the generation of fibrin at exposed subendothelial surfaces (77, 100). In purified experimental systems, the activation of factor X at limiting concentrations of tissue factor is markedly decreased in the absence of factor VIII or IX, suggesting that tissue-factor dependent coagulation proceeds largely through the activation of factor IX by factor VIIa when tissue factor concentration is limiting (77). These data form the basis for the hypothesis that limited tissue factor exposure at a damaged vessel wall, such as an arteriosclerotic coronary artery, could promote activation of factor X dependent on factors VII, IX, and VIII and lead to formation of a thrombus. Furthermore, some tissue macrophages can express tissue factor and perhaps factor VII, and there is strong evidence that macrophages derived from blood monocytes are involved in intimal proliferation and development of the arteriosclerotic plaque (87). This suggests the possible involvement of tissue factor and factor VII in development of the arteriosclerotic plaque as well as in the thrombotic component of ischemic heart disease, but at the moment this is purely speculative. The hypothesis that factor VII elevation is a risk factor for ischemic heart disease because of a thrombogenic mechanism underlies the proposal for an experimental trial of low dose warfarin in middle-aged men, in an attempt to reduce the incidence of ischemic cardiac events in this group (68). These investigators have determined that relatively small doses of warfarin can be used to reduce the factor VII level from the high-normal to the low-normal range. It is hoped that this therapy will

prove to be benign with regard to bleeding complications. This and similar therapeutic trials should provide further information as to the importance of the vitamin K-dependent clotting factors in the development of ischemic heart disease.

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